

=> d his

(FILE 'HOME' ENTERED AT 09:36:07 ON 20 OCT 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,  
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,  
CABA,  
CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,  
DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 09:36:14 ON  
20 OCT 2002

SEA (THERMOSTABLE CELLULASE)

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5 FILE AGRICOLA  
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186 FILE BIOTECHABS  
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10 FILE CABA  
62 FILE CAPLUS  
22 FILE CEABA-VTB  
1 FILE CIN  
1 FILE CONFSCI  
61 FILE DGENE  
14 FILE EMBASE  
11 FILE ESBIODASE  
6 FILE FEDRIP  
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L1 QUE (THERMOSTABLE CELLULASE)

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OCT FILE 'BIOTECHDS, CAPLUS, SCISEARCH, BIOSIS' ENTERED AT 09:39:10 ON 20  
2002

L2 FILE 'SCISEARCH, MEDLINE, EMBASE' ENTERED AT 09:39:51 ON 20 OCT 2002  
L3 7 S L1 AND (FAMILY 12)  
3 DUP REM L2 (4 DUPLICATES REMOVED)

=> d 13 ibib ab 1-3

L3 ANSWER 1 OF 3 SCISEARCH COPYRIGHT 2002 ISI (R)  
ACCESSION NUMBER: 2002:672352 SCISEARCH  
THE GENUINE ARTICLE: 581DU  
TITLE: The structure of *Rhodothermus marinus* Cell2A, a highly  
thermostable **family 12** endoglucanase,  
at 1.8 angstrom resolution  
AUTHOR: Crennell S J (Reprint); Hreggvidsson G O; Karlsson E N  
CORPORATE SOURCE: Univ Bath, Dept Biol & Biochem, Bath BA2 7AY, Avon,  
England (Reprint); Prokaria, Gylfaflot, Reykjavik,  
Iceland  
COUNTRY OF AUTHOR: England; Iceland  
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (19 JUL 2002) Vol. 320, No.  
4, pp. 883-897.  
Publisher: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD, 24-28  
OVAL RD, LONDON NW1 7DX, ENGLAND.  
ISSN: 0022-2836.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 56

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Cellulose is one of the most abundant polysaccharides in nature and  
microorganisms have developed a comprehensive system for enzymatic  
breakdown of this ubiquitous carbon source, a subject of much interest in  
the biotechnology industry. *Rhodothermus marinus* produces a hyper-  
**thermostable cellulase**, with a temperature optimum of  
more than 90degreesC, the structure of which is presented here to 1.8  
Angstrom resolution. The enzyme has been classified into glycoside  
hydrolase **family 12**; this is the first structure of a  
thermophilic member of this family to have been solved. The P-jelly roll  
fold observed has identical topology to those of the two mesophilic  
members of the family whose structures have been elucidated previously. A  
Hepes buffer molecule bound in the active site may have triggered a  
conformational change to an active configuration as the two catalytic  
residues Glu124 and Glu207, together with dependent residues, are  
observed  
in a conformation similar to that seen in the structure of *Streptomyces*  
*lividans* CelB2 complexed with an inhibitor. The structural similarity  
between this cellulase and the mesophilic enzymes serves to highlight  
features that may be responsible for its thermostability, chiefly an  
increase in ion pair number and the considerable stabilisation of a  
mobile  
region seen in *S. lividans* CelB2. Additional aromatic residues in the  
active site region may also contribute to the difference in  
thermophilicity. (C) 2002 Elsevier Science Ltd. All rights reserved.

L3 ANSWER 2 OF 3 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 1  
ACCESSION NUMBER: 1998:942580 SCISEARCH  
THE GENUINE ARTICLE: 145XB  
TITLE: Purification, characterization, and molecular analysis of  
**thermostable cellulases** Cella and CelB  
from *Thermotoga neapolitana*  
AUTHOR: Bok J D; Yernool D A; Eveleigh D E (Reprint)  
CORPORATE SOURCE: RUTGERS STATE UNIV, COOK COLL, DEPT MICROBIOL & BIOCHEM,  
76 LIPMAN DR, NEW BRUNSWICK, NJ 08901 (Reprint); RUTGERS  
STATE UNIV, COOK COLL, DEPT MICROBIOL & BIOCHEM, NEW  
BRUNSWICK, NJ 08901  
COUNTRY OF AUTHOR: USA

SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (DEC 1998) Vol. 64, No. 12, pp. 4774-4781.  
Publisher: AMER SOC MICROBIOLOGY, 125 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.  
ISSN: 0099-2240.

DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE; AGRI  
LANGUAGE: English  
REFERENCE COUNT: 54

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Two thermostable endocellulases, CelA and CelB, were purified from *Thermotoga neapolitana*. CelA (molecular mass, 29 kDa; pI 4.6) is optimally active at pH 6.0 at 95 degrees C, while CelB (molecular mass, 30 kDa; pI 4.1) has a broader optimal pH range (pH 6.0 to 6.6) at 106 degrees C. Both enzymes are characterized by a high level of activity (high V-max value and lent apparent K-m value) with carboxymethyl cellulose; the specific activities of CelA and CelB are 1,219 and 1,536 U/mg, respectively. With p-nitrophenyl cellobioside the V-max values of CelA and CelB are 69.2 and 18.4 U/mg, respectively, while the K-m values are 0.97 and 0.3 mM, respectively. The major end products of cellulose hydrolysis, glucose and cellobiose, competitively inhibit CelA, and CelB. The K-i values for CelA are 0.44 M bp glucose and 2.5 mM far cellobiose; the K-i values for CelB are 0.2 M for glucose and 1.16 mM for cellobiose. CelB preferentially cleaves larger celooligomers, producing cellobiose as the end product; it also exhibits significant transglycosylation activity. This enzyme is highly thermostable and has half-lives of 130 min at 106 degrees C and 26 min at 110 degrees C. A single clone encoding the celA and celB genes was identified by screening a *T. neapolitana* genomic library in *Escherichia coli*. The celA gene encodes a 257-amino-acid protein, while celB encodes a 274-amino-acid protein. Both proteins belong to family 12 of the glycosyl hydrolases, and the two proteins are 60% similar to each other. Northern blots of *T. neapolitana* mRNA revealed that celA and celB are monocistronic messages, and both genes are inducible by cellobiose and are repressed by glucose.

L3 ANSWER 3 OF 3 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 2

ACCESSION NUMBER: 1998:313351 SCISEARCH

THE GENUINE ARTICLE: ZH504

TITLE: Cloning, sequencing and overexpression of a *Rhodothermus marinus* gene encoding a **thermostable cellulase** of glycosyl hydrolase **family 12**

AUTHOR: Halldorsdottir S; Thorolfssdottir E T; Spilliaert R; Johansson M; Thorbjarnardottir S H; Palsdottir A; Hreggvidsson G O; Kristjansson J K; Holst O; Eggertsson G (Reprint)

CORPORATE SOURCE: UNIV ICELAND, INST BIOL, MOL GENET LAB, IS-108 REYKJAVIK, ICELAND (Reprint); UNIV ICELAND, INST BIOL, MOL GENET LAB,

IS-108 REYKJAVIK, ICELAND; TECHNOL INST ICELAND, DEPT BIOTECHNOL, IS-112 REYKJAVIK, ICELAND; LUND UNIV, CTR

CHEM

& CHEM ENGN, S-22100 LUND, SWEDEN

COUNTRY OF AUTHOR: ICELAND; SWEDEN

SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (MAR 1998) Vol. 49, No. 3, pp. 277-284.  
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.  
ISSN: 0175-7598.

DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE; AGRI

LANGUAGE: English  
REFERENCE COUNT: 36

\*ABSTRACT IS AVAILABLE IN THE ALL AVAILABLE FORMATS\*

AB A gene library from the thermophilic eubacterium *Rhodothermus marinus*, strain ITI 378, was constructed in pUC18 and transformed into *Escherichia coli*. Of 5400 transformants, 3 were active on carboxymethylcellulose. Three plasmids conferring cellulase activity were purified and were all found to contain the same cellulase gene, *celA*. The open reading frame

for

the *celA* gene is 780 base pairs and encodes a protein of 260 amino acids with a calculated molecular mass of 28.5 kDa. The amino acid sequence shows homology with cellulases in glycosyl hydrolase family 12. The *celA* gene was overexpressed in *E. coli* when the pET23, T7 phage RNA polymerase system was used. The enzyme showed activity on carboxymethylcellulose and lichenan, but not on birch xylan or laminarin. The expressed enzyme had six terminal histidine residues and was purified by using a nickel nitrilotriacetate column. The enzyme had a pH optimum

of

6-7 and its highest measured initial activity at 100 degrees C. The heat stability of the enzyme was increased by removal of the histidine residues. It then retained 75% of its activity after 8 h at 90 degrees C.